PB87-152567

Effects of Ground ULV (Ultra-Low Volume) Applications of Fenthion on Estuarine Biota. 1. Study Design and Implementation

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1985

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1. REPORT NO. EPA/600/J-85/464	2.	3. PB87-152567
4. TITLE AND SUBTITLE EFFECTS OF GROUND ULV APPLICATIONS OF FENTHION ON ESTUARINE BIOTA I. STUDY DESIGN AND IMPLEMENTATION (Journal Version)		5. REPORT DATE 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J.R. Clark*, B.P. Middaugh*, M.J. Hemmer*, B.W. Clements**, J.C. Dukes**, and C.B. Rathburn**		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS **Florida Dept. Health & Rehabilitative Services West Florida Arthropod Research Laboratory Panama City, FL 32406		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Gulf Breeze, FL Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, FL 32561		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/4

15. SUPPLEMENTARY NOTES

J. Fla. Anti-Mosquito Assn. 56(2):51-62

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 14
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

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EPA/600/J-85/464
JOURNAL ARTICLE

Reprinted from Journal of the Florida Anti-Mosquito Association Volume 56, Number 2, 1985

Effects of Ground ULV Applications of Fenthion on Estuarine Biota I. Study Design and Implementation[1]

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ABSTRACT

On the evenings of 5 May, 10 May, 13 June and 11 July, 1983, truck-mounted ULV equipment was used to apply fenthion at a rate of 11 g/ha to control adult salt marsh mosquitoes at two sites. Meterological conditions at the time of spray, spray droplet density, and droplet VMD were monitored. Mortality of caged *Aedes taeniorhynchus* and *Culex quinquefasciatus* was measured to evaluate spray effectiveness. Sand dunes and stands of pine trees affected wind speed and directions and reduced effectiveness during some sprays. When appropriate conditions prevailed, 100% mortality was achieved among caged mosquitoes placed up to 150 m from the spray source. These data provided the efficacy baseline for studies on nontarget species.

In order to test the field applicability of laboratory test methods used to generate hazard assessment data in support of pesticide registration, the U.S. Environmental Protection Agency's Environmental Research Laboratory at Gulf Breeze, Florida (ERL/BG) initiated a research program on pesticides applied under actual-use conditions to evaluate effects on nontarget, estuarine organisms. Chemical control of salt marsh mosquitoes provides pesticide use patterns from which direct exposure-response assessments can be made in the natural habitat of a variety of estuarine organisms. The series of articles (I-V) presents the results from our initial attempts to establish a field data base from four ULV applications of fenthion to control adult mosquitoes. This article provides an overview of the research program and its implementation; II discusses the chemical analyses of environmental samples; III and IV present results of caged estuarine animal studies, and V is a summary of benthic community monitoring and ancillary laboratory studies. Each article represents a discrete contribution to the field effort and should be interpreted within the context of the overall program.

[1]Contribution No. 523a Environmental Research Laboratory, Gulf Breeze, FL.

Fenthion (0,0-dimethly 0-[(4-methylthio)-m-tolyl] phosphorothioate) (Mobay Chemical Corporation) is an organophosphate insecticide registered as Baytex® for mosquito and fly control. In 1980-81, 53,150 l (14,040 gal) of this product were used by Florida Mosquito Control Districts (Florida Anti-Mosquito Association, 1981-82 Year Book). For our study, we choose ground ULV applications because this is the most common mosquito control method in Florida and it allows use of concentrated pesticides without carriers. Since larvicide applications of fenthion are not recommended in Florida, ground ULV applications to control adult salt marsh mosquitoes provided the best opportunity to obtain a direct pesticide application near estuarine habitats.

This insecticide is toxic to estuarine crustaceans. The label indicates that concentrations lethal to shrimp and crabs may be attained following applications at rates recommended for mosquito control. Applied at the maximum allowable rate of 112 g active ingredient per ha (0.10 lb/acre) over an estuarine system with an average of 0.3 m depth, the expected environmental concentration is 37 µg/l, assuming 100% fallout. This concentration exceeds the 96-hr LC_{50} for pink shrimp (*Penaeus duorarum*, 0.11 µg/l) and mysids

(*Mysidopsis bahia*, 0.15 µg/l) (Borthwick et al. 1985) and exceeds the 48-hr LC_{50} for grass shrimp (*Palaemonetes pugio*, 4.7 µg/l) (Borthwick et al. 1985) and blue crabs (*Calinectes sapidus*, 2.3 µg/l) (ERL/GB, unpublished data). Acute effects on juvenile estuarine fishes are not to be expected following such mosquito control applications of fenthion since the 48-hr LC_{50} values for inland silversides, *Menidia beryllina*, and sheepshead minnows, *Cyprinodon variegatus*, are 2,140 µg/l and 1,890 µg/l, respectively (Borthwick et al. 1985), and for striped mullet, *Mugil cephalus*, and spot *Leiostomus xanthurus*, the 48-hr LC_{50s} are 1,600 µg/l and 1,200 µg/l, respectively (ERL/GB, unpublished data).

Field studies on the effects of mosquito control applications of fenthion on nontarget, aquatic organisms have employed low-volume aerial treatments or larvicide doses; however, we found no studies of ground ULV applications in our literature review. Linn (1968) found no effects on green sunfish, *Lepomis cyanellus*, following low-volume aerial applications of fenthion at 112 g/ha (0.1 lb technical/acre). Larvicide applications of fenthion (25% emulsifiable concentrate) to freshwater ponds at 112 g/ha or 448 g/ha (0.1 or 0.4 lb technical/acre) produced 6% and 18% mortality, respectively, in caged mosquito fish, *Gambusia affinis*, after 48 hr (Mulla, 1961). Applications of granular formulations of fenthion at 224 g/ha (0.2 lb technical/acre) to test ponds were toxic to midge larvae and cladocera, but produced no overt toxic effects on copepods, ostracods, hydra, annelids, snails, clams or mosquito fish (Patterson and von Wideguth, 1964). The findings of these field studies agree with laboratory toxicity data, indicating that many crustaceans are much more sensitive than fish (Johnson and Finley, 1980; Patterson and von Windeguth, 1964), although none of the field studies reported measured water concentrations of fenthion that would allow direct comparison.

Two studies on applications of granular formulations (5%) of fenthion at 224 g/ha to salt marsh habitats by Wall and Marganian (1971, 1973) produced overt mortality in Collembola and fiddler crabs and reductions in numbers of amphipods and tanaidaceans in test plots. No effects were noted on caged welks, *Nassarius obsoletus*, clams, *Mya arenaria*, horseshoe crabs, *Limulus polyphemus*, and fishes, *Fundulus spp.*, *Syngnathus fuscus*, and *Brevoortia tyrannus* during the first study. In the second study, no effects were noted on caged prawns, *Palaemonetes* sp., and fiddler crabs, *Uca pagnax*, deployed in an area adjacent to the treated plot; however, one of three caged mummichogs, *Fundulus heteroclitus* (the only fish caged in the study), died. The greater sensitivity of estuarine crustaceans to fenthion relative to fishes reported by Borthwick et al. (1985) is substantiated by the deaths of fiddler crabs and general lack of response of fishes reported by Wall and Marganian (1971, 1973). Again, however, the lack of measured exposure concentrations in the field prohibits direct comparison.

The recommended ground ULV application rate of 11 g/ha (0.01 lb technical/acre) will yield an expected environmental concentration of 3.7 µg/l, assuming 100% deposition in an acre-foot of water, which exceeds the shrimp and crab 96-hr LC_{50} concentrations. ULV applications of fenthion for control of adult mosquito populations are intended to produce an aerial "concentration" of pesticide to pass through the habitat of target species and enter aquatic habitats only as droplets settle or are blown onto the water surface. Environmental concentrations and animal responses should be different than those reported following direct, larvicide treatments with fenthion.

The objective of the research reported in this article was to establish that our field research program was based on efficacious applications of fenthion under typical use conditions. The goals of our field studies research program were to obtain empirical data on environmental concentrations attained following actual-use applications of fenthion, to ascertain responses of caged animals in the field, to assess the impact on benthic populations, and to compare all field results with data from routine laboratory tests. The combined results of our research should be interpreted largely as site-specific because dilution and flushing rates at the study sites controlled exposure concentrations and thereby influenced animal responses. Also, meteorological conditions at the time of pesticide application greatly influenced the amount of

chemical deposited in the estuarine system. However, some broad generalities in the results are pertinent to all mosquito control programs, such as the magnitude of initial water concentrations versus predicted concentrations based on application rates and the comparability of animal responses in the field with laboratory predictions of effect concentrations. Eventually, we will develop a field data base sufficient to evaluate the role of specific and general factors which affect how pesticides enter and are distributed within estuarine ecosystems and how these factors relate to the adequacy of laboratory data used in pesticide registration.

METHODS AND MATERIALS

Three estuarine water bodies surrounded by marsh grass, *Juncus roemerianus*, within the Eglin Air Force Base Reservation on Santa Rosa Island, Florida, were selected for study. Each site was on the north side of an east-west road used by the spray vehicle. Two received ground ULV applications of fenthion, the third was not treated and served as a reference area for deployment of control animals.

Contour maps of the two sites exposed to fenthion were prepared (Figures 1 and 2) to aid in calculating surface area and water volume. Elevation contours are given in meters above or below the mean low water datum. The locations of sampling stations for water samples and caged animals are shown also. The reference site (not illustrated) was 4.8 km (3 mi) east of Site 1 and 11 km (6.8 mi) east of Site 2. Baseline data on minimum-maximum water temperatures, salinity at each site and adjacent Santa Rosa Sound, and amounts of rainfall were collected at weekly intervals from March through August, 1983, at each site.

Site 1 (Figure 1) was located between sand dunes (10 m high) on the east and small dunes (<1 m) and *Juncus* on the west. A stand of pines with 3 m dunes was to the south, opposite the road. Water from Site 1 exchanged freely with Santa Rosa Sound (to the north) with each tide. This site also was influenced by surges and waves generated by boat traffic in the sound. The water's surface area, from the edge nearest the road to approximately 65 m north where it began to open widely into the sound, was 0.22 ha (0.55 acres) at mean low tide. Site 1 contained 3 bottom types: (1) fine detritus associated with the pool between stations A and B, (2) coarse, shifting sand forming a sand bar between B and C, and (3) a combination of fine detritus and sand along the perimeter of the pond.

Site 2 (Figure 2) was situated within a broad open area of low dunes, rain catchment ponds, and marsh grass. At this site, water exchanged with Santa Rosa Sound on a limited basis through a small tidal creek. Site 2 drained a 5.6 ha (13.8 acres) *Juncus* marsh. The surface area of the pond at low tide was 0.2 ha (0.45 acres). At high tide, the edge of the flooding waters was not always discernable within the *Juncus* marsh. Composition of the substrate changed gradually along a longitudinal transect, from a layer of fine detritus more than 1 m deep along the southeastern edge of the pond to organically enriched coarse sand at the northern perimeter. Because of the limited exchange with the sound and the highly organic bottom, nightly dissolved oxygen concentrations at Site 2 frequently fell to levels that would not sustain caged animals deployed overnight. Thus, observations on responses of caged animals to ground ULV applications of fenthion will not be reported from this site.

The reference site was a tidal creek that connected Pirates' Cove, a 4.2 ha (10.4 acre) lagoon, and Santa Rosa Sound. The bottom along the tidal creek was firm sand and shell. Water quality at the reference site was similar to that of Santa Rosa Sound, and always was sufficient to sustain caged animals.

Fenthion (Baytex®, 93% liquid concentrate) was applied at dusk with a truck-mounted, Leco® HD ultra low volume (ULV) aerosol generator (nonthermal) on the evenings of 5 May, 10 May, 13 June, and 11 July 1983. Applications were at the recommended rate of 30 ml (1 fl. oz.) per minute with a truck speed of 16 km/hr (10 mph) and swath width of 91.5 m (300 ft) (Rathburn 1977), using methods similar to those of Rathburn and Boike (1975). This is equivalent to an application rate of 11 g/ha (0.01 lb per acre). The two treated sites were sprayed from the road along the south end of the salt marsh areas using a

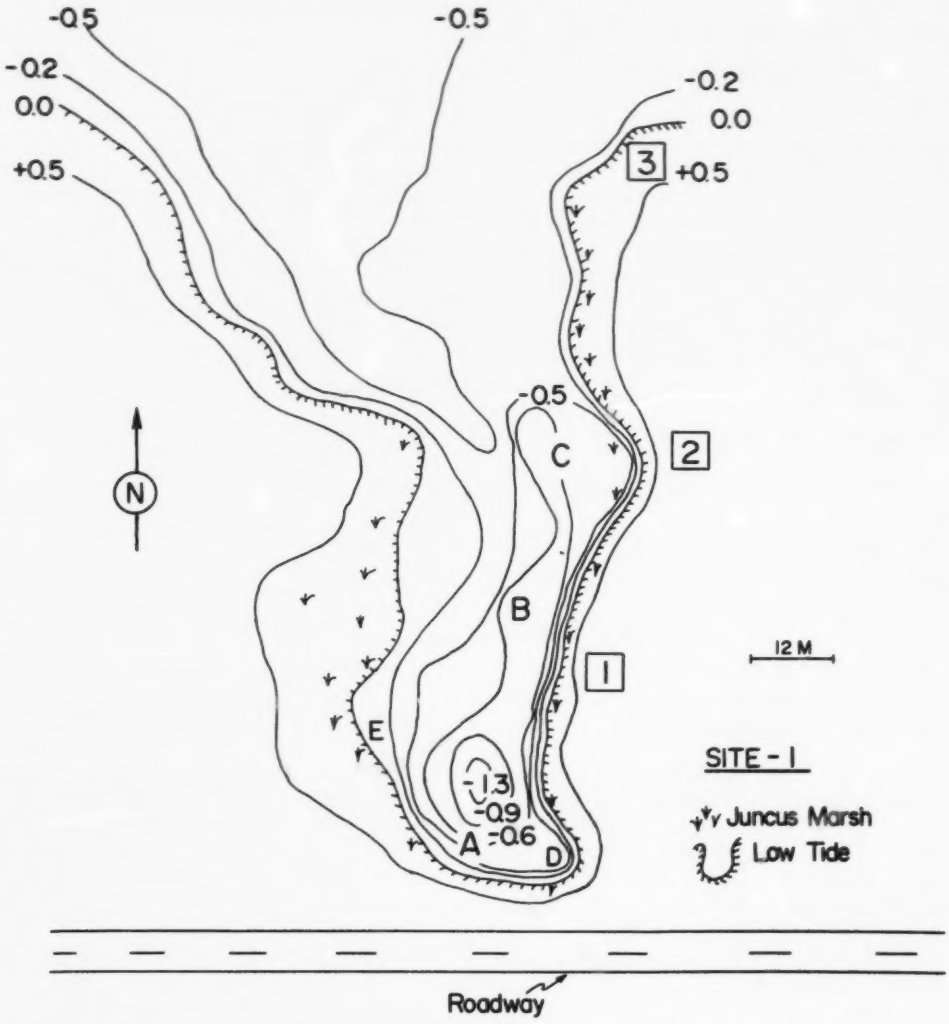

Fig. 1. Site 1 on Santa Rosa Island within the Eglin AFB Reservation. The site is bordered on the north by Santa Rosa Sound and on the south by a roadway utilized for truck-mounted ULV application of fenthion. Elevations are in meters above or below mean low tide. A-E indicate water sampling stations. Estuarine animals were caged at stations A, B, and C; caged mosquitoes were placed at stations 1, 2, and 3.

single pass of the spray truck. Wind speed and direction, air temperature, and relative humidity were monitored during pesticide application.

Adult *Aedes taeniorhynchus* and *Culex quinquefasciatus* 3 to 7 days post-emergence were deployed in screened cages (15 cm diameter, 2.5 cm deep, with 14 × 18 mesh screen) with 25 female mosquitoes per cage at 3 stations along the east margin of the study sites, dividing the study area into thirds (Figures 1 and 2). Cages were approximately 45 m, 76 m, and 120 m from the road at Site 1 and 45 m, 100 m, and 150 m from the road at Site 2. One group of cages was deployed 45 m from the road at the Reference Site. Prior to each application, two cages of each species of mosquito (four cages total) were placed at each station on support rods at the height of the *Juncus* grass or up to 1 m above it. Thirty minutes after the spray truck passed, the caged mosquitoes were retrieved, anesthetized with carbon dioxide, transfer-

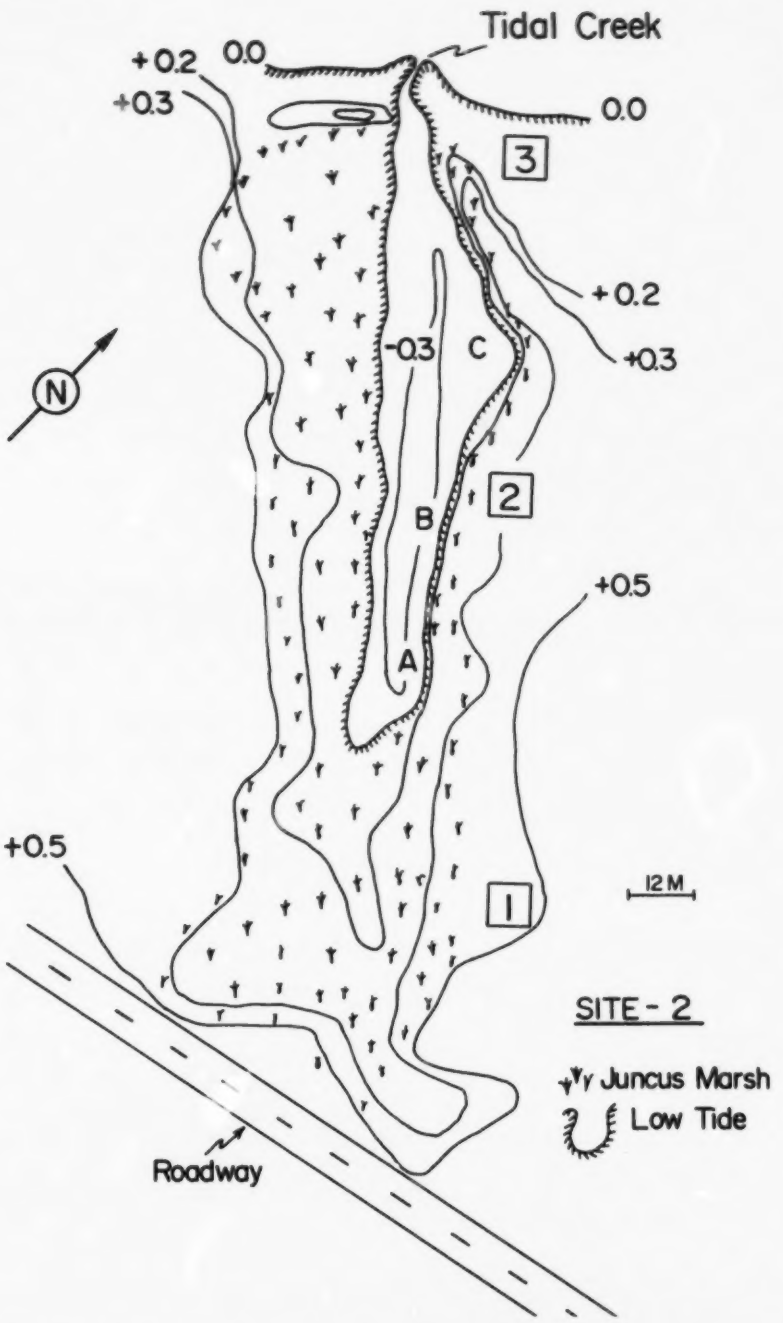

Fig. 2. Site 2 on Santa Rosa Island within the Eglin AFB Reservation. The site is bordered on the northeast by Santa Rosa Sound and on the southwest by a roadway utilized for truck-mounted ULV application of fenthion. A wide expanse of *Juncus* marsh separates the road and open water habitat at this site. Elevations are in meters above or below mean low tide. A-C indicate water sampling stations and caging stations for estuaring animals; 1, 2, and 3 indicate caged mosquito stations.

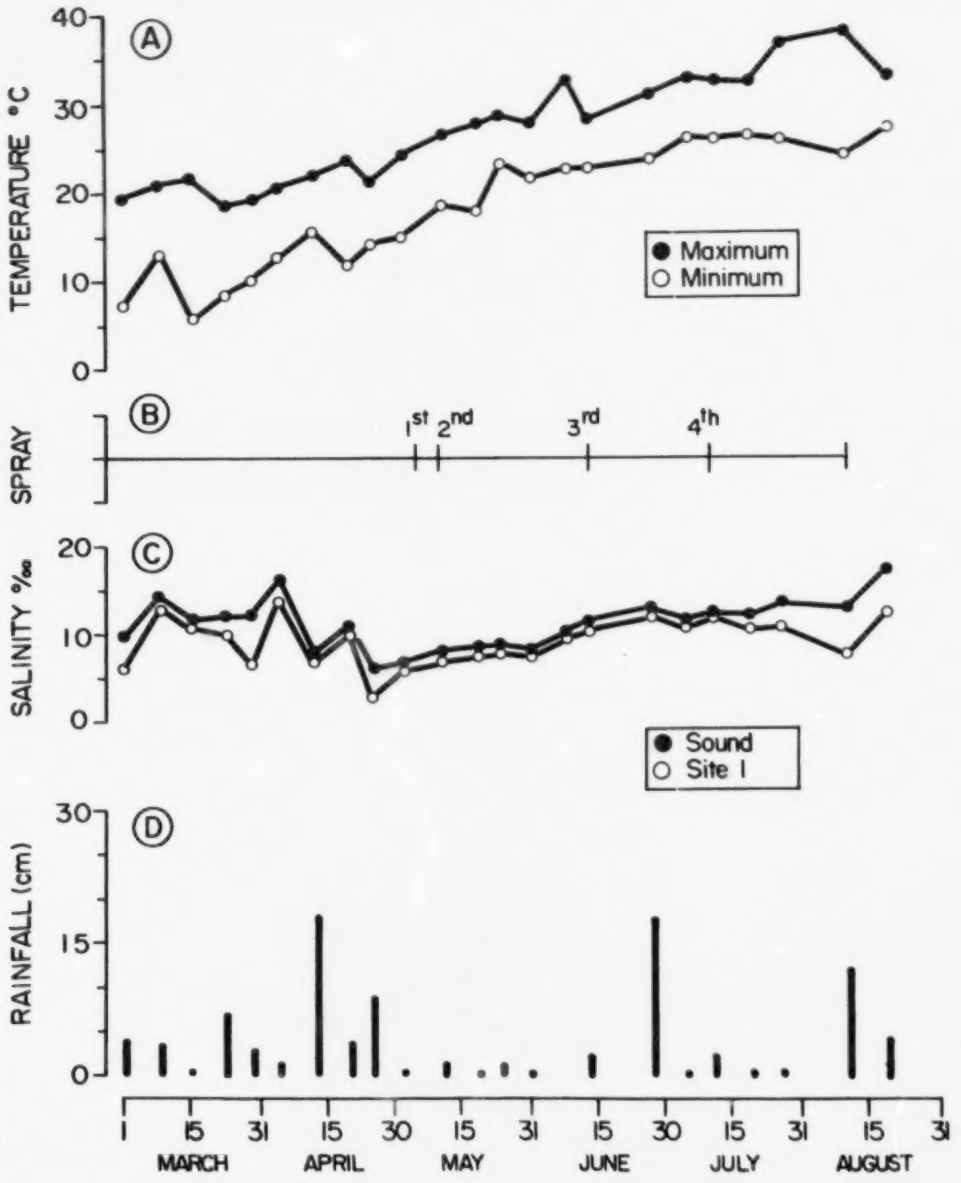

Fig. 3. Environmental data for Site 1. A—ambient water temperatures, B—spray schedule, C—ambient salinities, and D—rainfall. Salinities from Santa Rosa Sound provided for reference.

red to clean holding cages with access to a 10% sugar solution, and transported to the laboratory. The next morning, dead mosquitoes were counted and percentage killed computed for each station, with adjustments for mortality of controls. Droplets were collected on teflon-coated glass slides rotated for 5 min on an impactor at 3.6 m/sec. When sufficient numbers were obtained, droplet density and volume median diameter (VMD) were calculated (Rathburn, 1970).

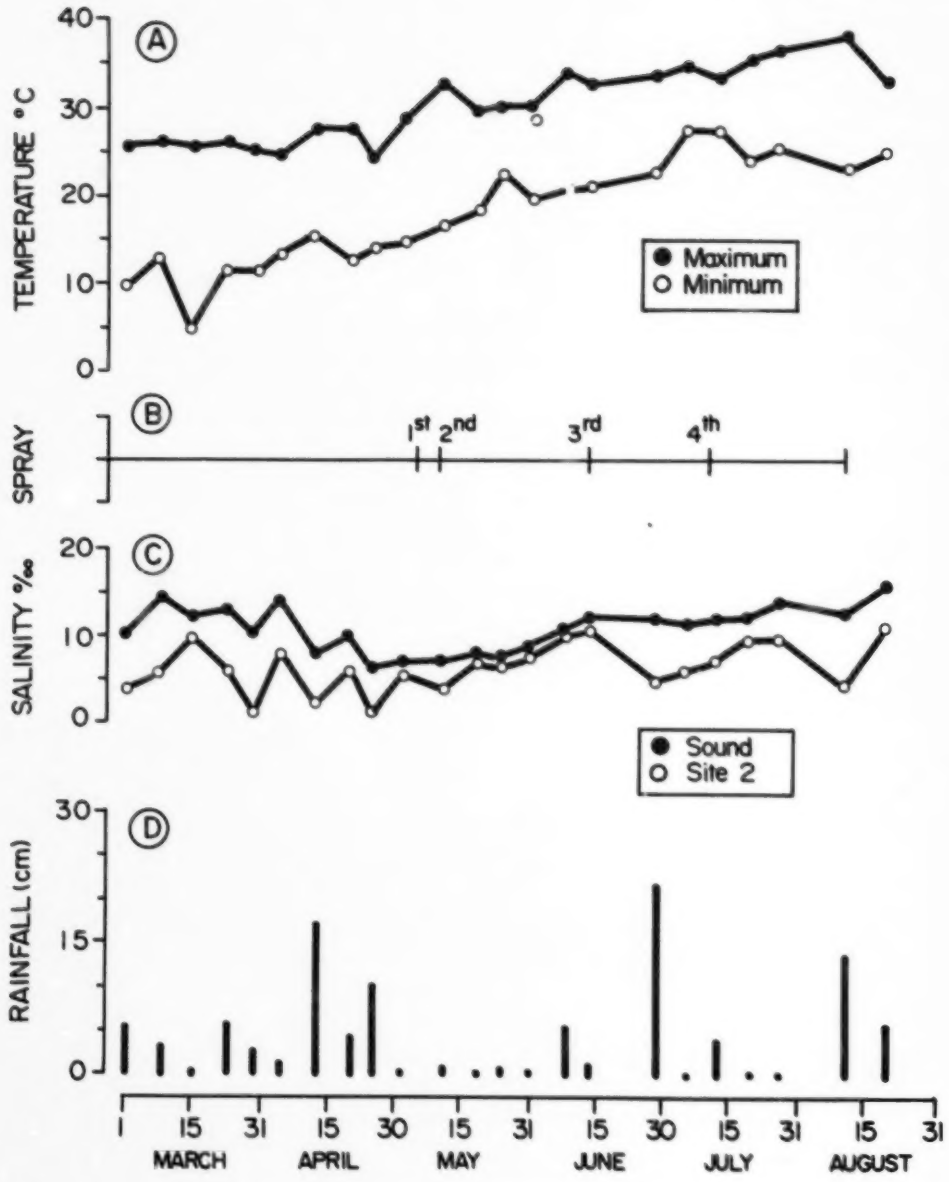

Fig. 4. Environmental data for Site 2. A—ambient water temperatures, B—spray schedule, C—ambient salinities, and D—rainfall. Salinities from Santa Rosa Sound provided for reference.

RESULTS AND DISCUSSION

The physical measurements taken at the sprayed sites were similar to those at the reference area, although some differences occurred in water temperature, salinity and rainfall (Figures 3,4,5). Each site experienced the expected seasonal rise in minimum and maximum temperatures. Santa Rosa Sound salinities were similar at the Reference Site and Site 1; however, salinity at Site 2 was usually 3 to 5 parts per

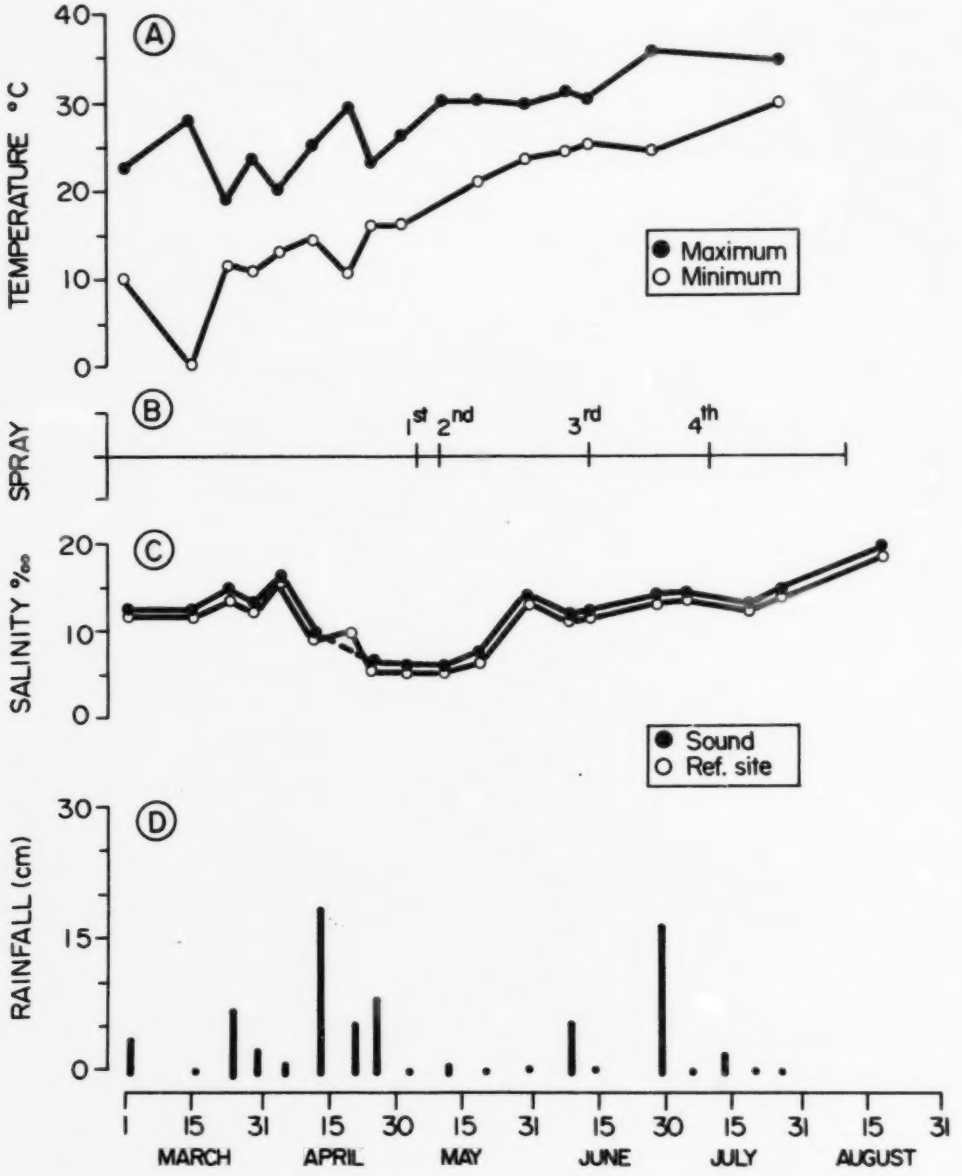

Fig. 5. Environmental data for the Reference Site. A—ambient water temperatures, B—spray schedule, C—ambient salinities, and D—rainfall. Salinities from Santa Rosa Sound provided for reference.

thousand lower than adjacent sound waters. Rainfall during the spring and summer of 1983 was very high; through August it totalled 148 cm, a value 58 cm above normal.

Applications of fenthion were made at dusk, the time of greatest mosquito activity and optimal wind conditions. Southerly sea breezes, generated during the day by thermal stratification at the land-sea interface, blew from the Gulf of Mexico at velocities from 3.2 to 12.8 km/h to provide transport of the spray droplets across the marsh habitat. Application was delayed

Table 1. Meteorological conditions at time of ground ULV applications of fenthion and monitoring of application rate.

Test Date	Spray No.	Site	Time of Spray Start-End	Actual dischg. ml/min	Wind km/hr	Wind Direction[1]	Air Temp. °C	Rel. Humid. %
5 May 83	1	1	1950-1955	30	8.5	45-90°	—[2]	—
		2	2030-2038	30	—	45-90°	—	—
10 May 83	2	1	1930-1935	27	0.6	0-45°	23	85
		2	1945-1950	33	4.2	0-45°	24	—
13 June 83	3	1	1945-1950	33	3.0	0-45°	26	94
		2	1915-1920	33	5.0	0-45°	26	94
11 July 83	4	1	1915-1920	30	8.8	0°	30	—
		2	1940-1945	30	8.8	0°	30	—

[1]Wind directions noted as degrees deviating from direction of spray nozzle which was perpendicular to road. (Refer to site figures.)
[2]— indicates no measurements taken.

Table 2. Mortality of caged mosquitoes subjected to ground ULV applications of fenthion with characterization of spray droplets.

Spray No.	Site No.	Station No.	Distance Meters[1]	Average[2] percentage kill		Droplet VMD (μm)[3]	Avg. No. Droplets per traverse[3]
				Aedes	Culex		
1	1	1	45	96.5	98.1	—[4]	—
		2	76	58.3	76.6	—	—
		3	120	36.9	62.7	—	—
	2	1	45	26.7	63.6	—	—
		2	100	5.9	4.3	—	—
		3	150	0	22.0	—	—
2	1	1	45	97.9	100	—	—
		2	76	86.3	97.1	13.3	19
		3	120	90.6	100	12.9	14
	2	1	45	98.0	100	—	—
		2	100	87.8	95.8	13.1	6
		3	150	98.0	100	12.9	8
3	1	1	45	32.7	1.6	—	—
		2	76	8.7	7.2	I.D.	2
		3	120	4.0	7.5	10.1	5
	2	1	45	100	100	—	—
		2	100	57.9	20.6	I.D.	2
		3	150	93.6	80.3	11.3	7
4	1	1	45	100	100	—	—
		2	76	100	100	9.0	5
		3	120	100	100	I.D.	3
	2	1	45	100	100	9.0[5]	17[5]
		2	100	100	100	I.D.	2
		3	150	100	100	7.7	14

[1]Distance is meters perpendicular from path of ULV generator.
[2]Corrected for control mortality which averaged 4.0%, 0.3%, 9.1%, and 3.6% for the four sprays respectively for *Aedes taeniorhynchus* and 0%, 0%, 0.3%, and 4% for the four sprays with *Culex quinquefasciatus*.
[3]One traverse was approximately 45.0 × 0.34 mm. A minimum of 5 traverses on each of two slides were counted to determine VMD. Missing data for Spray No. 1 were due to malfunction of droplet impactors. I.D. denotes insufficient droplets sampled to determine VMD. Droplet samples were not taken at station No. 1 for any spray test.
[4]— indicates no measurements taken.
[5]Samples collected at 5 m perpendicular to path of ULV generator.

until sunset when the soil had begun to cool to reduce the effects of upwelling air currents over warm land masses. Tidal stage was noted at the time of spray; however, we did not schedule the applications at a particular point in the tidal cycle.

Table 1 summarizes pertinent details of pesticide application and meteorological conditions. Because wind speed and direction and air temperatures were different during each spray and our data set is limited to only four spray operations, attempts to assess the relative importance of each variable to mosquito kill or measured water concentrations of pesticide through statistical analyses would be pointless and were beyond the scope of our experimental design. However, environmental factors were considered when evaluating the efficacy of each spray and are discussed in light of prevailing conditions at the time of spray.

Wind speeds and directions varied among sites on a given evening due to local land contours and vegetation. During Sprays 1 and 3, the tall dunes and trees at Site 1 funneled the prevailing winds to carry the spray droplets away from the pond area and caged mosquito sites, resulting in low mortalities in the target species. The wind speeds were lower at Site 1 due to natural barriers, except during Spray 4 when winds came directly from the south and sustained velocities that were equal to those at Site 2.

Mortality of caged mosquitoes at the monitoring stations varied between sites and among stations within a site (Table 2). Mortality from the 5 May spray (#1) at Site 1 decreased from 96% *Ae. taeniorhynchus* and 98% *Cx. quinquefasciatus* at the station closest to the road to less than 37% and 63%, respectively, in the mosquito cages 120 m from the spray source. At Site 2, the west-southwest winds did not carry fenthion droplets across the marsh to produce high mortality in the caged mosquitoes. Only 26.7% of the *Aedes* and 63.6% of the *Culex* mosquitoes were killed at the station near the road and less than 25% were killed at the other two stations. On 10 May (Spray 2), the light southwesterly winds transported fenthion droplets slowly over the marshes, producing an effective kill at both sites ($\geqq$ 86.3% killed for both species) at distances up to 150 m from the road. In June (Spray 3), more than 90% of the mosquitoes deployed in cages at Site 1 survived the spray, indicating that the dunes and vegetation protected them from spray droplets carried by southeasterly winds; whereas more than 80% of the mosquitoes were killed at 2 of the 3 stations monitored at Site 2. During Spray 4, with winds directly out of the south at 8.8 km/h, 100% mortality occurred in all mosquito cages at both sites.

The recommended maximum VMD for ULV applications of fenthion is 15 μm. All characterizations indicated the maximum VMD was not exceeded (Table 2). Estimates of numbers of droplets per traverse indicated no clear trends in the dispersal of the ULV spray as it passed through the salt marsh habitats.

The effectiveness of ground ULV applications of fenthion to control salt marsh mosquitoes varied from date to date and site to site. Application equipment could not be positioned in areas more appropriate for prevailing wind conditions because of the lack of alternate roads at the study sites. Greater mortality in caged mosquitoes on a given evening may have been obtained with less than optimal wind conditions if repeated applications had been made or if alternate roads had been available. Data characterizing the mortality of caged mosquitoes provide evidence that nontarget animal studies and analytical chemistry results, which are reported in subsequent papers in this series, are based on efficacious applications of fenthion.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of the U.S. Air Force and The Command of The Eglin Air Force Base Armament Division for providing a Field Study Site. Lt. Col. Richard Hartman of the Environmental Protection Division, Eglin Air Force Base, provided essential support and coordination for our study at a secured site on the Eglin AFB Reservation. The organizational and editorial assistance of J. Lowe, G. Walsh, D. Weber and N. Cooley and typists M. Stubbs and V. Caston are acknowledged.

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